Pathophysiology of Wound Healing

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Tissue injury stimulates an ordered cascade of events, commencing with coagulation, through inflammation and culminating in repair. Whereas the initial responses are similar, subsequent events depend on the type of tissue as well as the degree of damage. Wound healing largely involves repair by replacement with connective tissue. Repair can be achieved by either resolution, regeneration or replacement (with scar tissue, sometimes called organization).

Many cell types and inflammatory mediators such as cytokines are implicated in the repair process. In addition, there are both intrinsic and extrinsic factors that influence the way in which wounds heal. Striking differences between foetal and adult wound healing have recently been discovered and further research in this area may lead to improvements in our understanding and management of healing tissue.

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Repair by regeneration

For tissues to undergo repair by regeneration they must consist of cells that are either resident within the cell cycle and therefore continually dividing (i.e. labile cells) or cells that are in a state of quiescence but capable of re-entering the cell cycle (i.e. stable cells). Labile cells are typically epithelial cells, as in the skin and gastrointestinal tract, or lymphohaematopoietic cells. Stable cells include epithelial cells from solid glandular organs, and connective tissue cells such as fibroblasts, smooth muscle cells, osteoblasts, chondroblasts and endothelial cells.

Ultimately, whether regeneration can occur is governed by the extent of tissue destruction. If the structural framework, i.e. the basement membrane, of a tissue is disrupted, then repair can occur only by replacement with connective tissue. Similarly, damage to permanent cells (i.e. those incapable of further division) such as nervous tissue, skeletal or cardiac muscle can be repaired only by replacement. There are few examples where a surgical wound will not cause the degree of tissue damage that requires repair by replacement with connective tissue.

Resolution versus organization

Resolution occurs when tissues have been inflamed without subsequent cellular destruction and where any inflammatory exudate has been removed; the end result is that the tissue is in the same state of health as before the inflammation. If an inflammatory event occurs without the full removal of the inflammatory exudate and is accompanied by tissue destruction, then that exudate is replaced with connective tissue rich in collagen and small blood vessels (granulation tissue). This eventually matures into a scar; the process is then known as organization. When tissue destruction following mechanical injury is marked, the same connective tissue rich in collagen and vessels is deposited within the wound space. Over time this tissue matures into a fibrous scar – the classic method of repair by replacement.

Overview of the inflammatory response

Inflammation is a protective response: for example, it localizes and prevents the spread of damaging agents such as bacteria. If tissue damage occurs, inflammation is followed by repair, so it can be seen that the processes are closely linked. The inflammatory reaction is split into several phases: haemodynamic disruption, margination, adhesion, migration, and phagocytosis and degradation.

Haemodynamic disruption: following injury, transient vasoconstriction of arterioles occurs within a tissue. However, vasodilation soon predominates, giving rise to the classical features of heat and redness (calor and rubor) described by the Roman, Cornelius Celsus. An increase in hydrostatic pressure in capillaries, as a consequence of arterial vasodilation, causes an initial, protein-poor fluid transudate to collect in the extracellular space. A gradual increase in small vessel permeability, however, causes exudation of protein-rich fluid, which contributes to tissue oedema or swelling (tumour), one cause of the pain (dolor) associated with injury. The loss of fluid increases blood viscosity, disrupts laminar flow in post-capillary venules and eventually leads to intravascular stasis.

Margination: this normally occurs in the post-capillary venules. The disruption to laminar blood flow in the microcirculation, as mentioned above, encourages the heavier leukocytes to align peripherally in the column of blood, allowing them to come into contact with the endothelium.

Adhesion: these marginated leukocytes will adhere to the endothelium. This is a complex mechanism involving specific interaction between adhesion molecules present on both the leukocyte and endothelial surface. Initial adhesion is mediated by selectins. Tethering is weak and, in the absence of strong adhesion, leukocytes soon tear free. In response to pro-inflammatory mediators such as chemokines (short for chemotactic cytokines) leukocytes become activated and express integrins on their cell surface which allow strong adhesion.

Migration: following strong adhesion, activated leukocytes manoeuvre between the widened endothelial cell junctions (caused by the increase in vessel permeability) to accumulate in the extracellular space. This process is also under the control of members of the chemokine family, which are immobilized in the extra-

cellular matrix and provide a chemotactic gradient along which leukocytes migrate.

Phagocytosis and degradation: bacteria, debris and foreign material are first recognized and then engulfed by neutrophils and macrophages prior to killing and degradation. This involves degranulation of lytic enzymes in the phagosome. In addition, extracellular degranulation of vesicles occurs, allowing secretion of various factors subsequently involved in the ongoing inflammatory response and healing of the affected tissues.

Repair and organization

Healing by first intention: healing by first intention (primary union) is the way most surgical wounds heal. Typically, such wounds are created in aseptic conditions with minimal bacterial contamination and a minor amount of tissue damage. They have accurately opposed and sutured wound edges. Wounds lacking these features are unlikely to heal by first intention.

0–48 hours post injury – Bleeding is the first response following injury. Platelets attracted in this process promote haemostasis by accelerating fibrin deposition and formation of a platelet plug. Platelets also release cytokines (Figures 1 and 2), which recruit neutrophils, and include the chemokines interleukin-8 (IL-8), platelet factor-4 (PF-4) and β-thromboglobulin (β-THG), as well as transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF). Neutrophils are not required for normal repair in the absence of infection but aid the initial removal of the fibrin clot. After 24 hours, monocytes start to infiltrate the wound, largely under the influence of the cytokines TGF-β and PDGF and the chemokine, monocyte chemotactic protein-1 (MCP-1). Monocytes have two primary roles within the wound: first, to continue clearing debris from the site of injury, and second, to produce further cytokines which attract those cell types capable of laying down granulation tissue, that is, fibroblasts and endothelial cells. During this period there is also proliferation of epithelial cells at the epidermal–dermal junction, which migrate toward the midline re-forming a thin epidermal layer under the surface clot.

3–5 days post injury – Epithelial proliferation (epithelialization) continues beneath the surface clot (scab) with subsequent surface keratinization. Keratinocyte growth factor (KGF) – an alternate designation is fibroblast growth factor-7 (FGF-7) – is secreted by dermal cells, and has been implicated in epithelial proliferation (Figure 1).

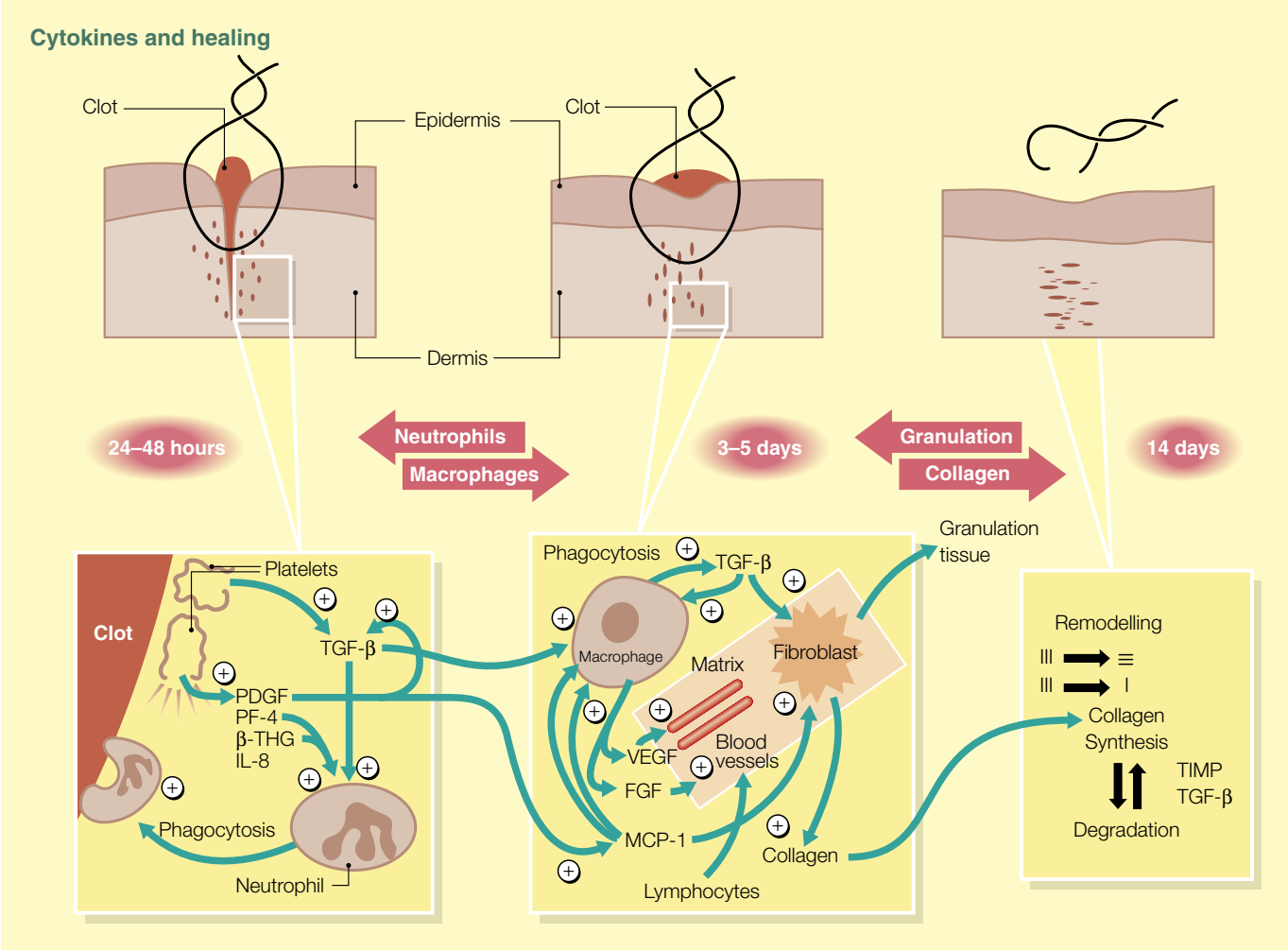
This phase of healing is also typified by the gradual appearance of granulation tissue, which consists of newly developing blood vessels with surrounding fibroblasts and additional elements of extracellular matrix, creating a pink, velvety appearance from which the name arises. Collagen deposition from fibroblasts is largely under the control of TGF-β, whereas neovascular development is stimulated by vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) – all factors secreted by monocytes. Towards the end of the fifth day, neovascularization is maximal with a gradual increase in the amount of granulation tissue, which bridges the defect.

2 weeks post injury – Granulation tissue begins to be remodelled and its

Cytokines involved in wound healing

		Cell source	Effect
Interleukin-8	IL-8	Platelets Macrophages Endothelial cells	Chemotactic (*N*)
Platelet factor-4	PF-4	Platelets	Chemotactic (*N*)
β-thromboglobulin	β-THG	Platelets	Chemotactic (*N*)
Platelet-derived growth factor	PDGF	Platelets	Chemotactic *(N,F,M)* ↑ Integrin expression ↑ MCP-1 synthesis ↑ TGF-β synthesis
Transforming growth factor-β	TGF-β	Platelets Macrophages	Chemotactic *(F,M)* ↑ Collagen deposition ↑ TIMP-1 synthesis
Monocyte chemotactic protein-1	MCP-1	Macrophages Fibroblasts Endothelial cells	Chemotactic *(M)*
Basic fibroblast growth factor	BFGF	Matrix Macrophages	Chemotactic *(E)* Angiogenic
Vascular endothelial growth factor	VEGF	Macrophages	Chemotactic *(E)* Angiogenic

N = neutrophil, *M* = macrophage, *F* = fibroblast, *E* = endothelial cell



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vascularity decreases as the amount of collagen increases. Maturation of the scar occurs over the next few months and is characterized by further remodelling. Collagen produced from fibroblasts is initially laid down in a vertical manner, but gradually changes its orientation to align across the defect, leading to increased wound strength. In addition, collagen type III which is initially laid down in the immature scar is replaced with the more mature collagen type I.

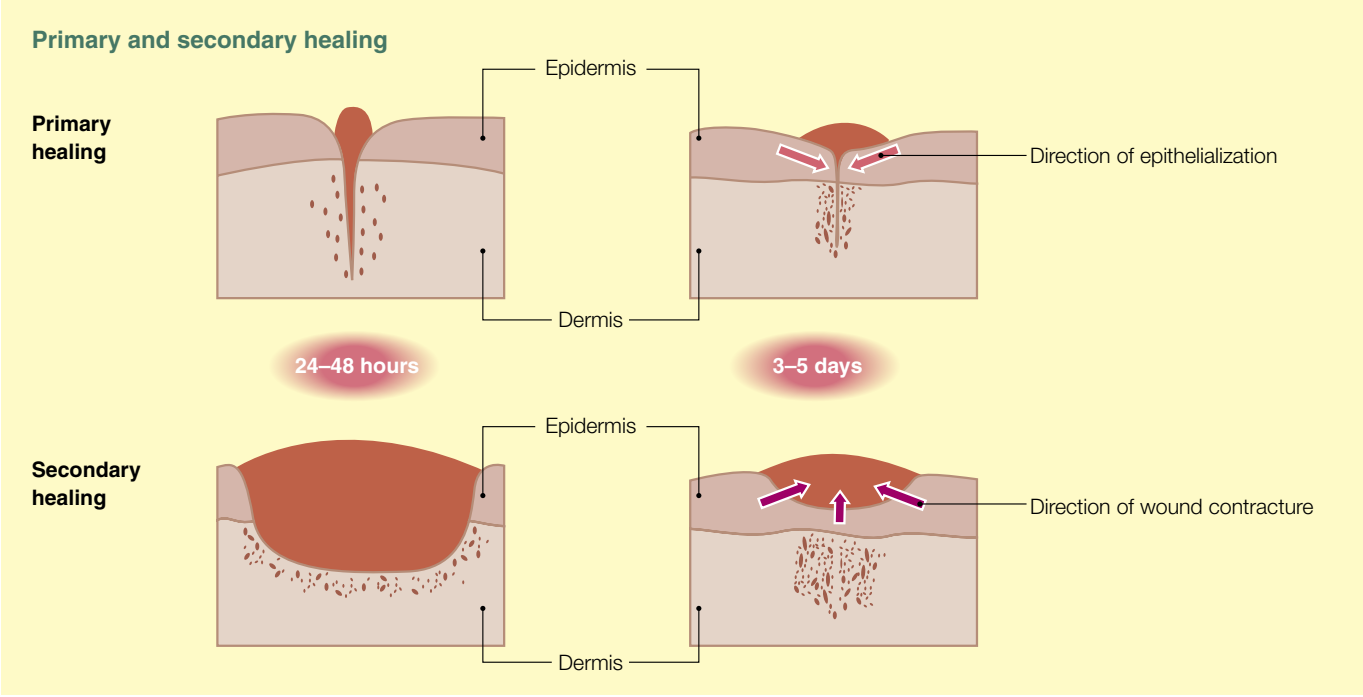
A healed skin wound will never achieve the tensile strength found previously in undamaged skin. Following wounding, the initial strength of skin is provided only by the sutures and only 10% of the original tissue strength is regained one week following injury. However, after this initial lag phase, there is an increase in wound strength. At around one month following injury approximately 70–80% of the initial strength has returned. This gain in strength is partly attributable to increased collagen deposition early in wound healing, but probably more importantly due to the later remodelling of collagen discussed above.

Healing by second intention: there are three main reasons why wounds will undergo this form of healing: wound contamination or infection, substantial tissue damage or lack of skin edge opposition. This form of repair is also encountered following ulceration, abscess formation, major superficial wounds or tissue infarction.

The order of events in healing by second intention (secondary union) is similar to that found in healing by first intention and the processes are essentially the same. Differences are related to the degree of the inflammatory response and subsequent organization. Tissue damage is often greater and, in the presence of contamination, there is an exaggerated inflammatory response in order to clear debris. There is subsequently an increased amount of granulation tissue deposition to bridge the tissue defect and to aid eventual repair (Figure 3). An important factor that aids secondary healing is wound contracture. Myofibroblasts (fibroblasts that have acquired characteristics of smooth muscle cells) have been implicated in this process and are capable of reducing a surface defect to 5–10% of its original size. Epithelialization is most rapid in moist conditions, which is the principle underlying many occlusive dressings in use today.

Collagenization

Collagen is a major component of extracellular connective tissue and is important in giving support and substance to the healed wound, along with other matrix proteins such as glycosaminoglycans. The metabolism of collagen is dynamic, there being a constant flux between synthesis



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and degradation. This allows collagen remodelling, which is crucial to the development of tensile strength across the healing wound.

Collagen molecules (tropocollagen) consist of three α chains (polypeptide chains) woven into a triple-stranded, left-handed helix. Each α chain has a highly repetitive sequence of amino acids, with almost every third residue being glycine. However, there are slight differences in the α chains allowing 11 different collagen types to be recognized. Only collagen types I–III form fibrils, with types IV–XI contributing to the amorphous substance of basement membrane and interstitial tissue. Fibrillar collagen rods are 300 × 1.5 nm in size and have a mass of ≈ 285kD. When these are joined together the resultant 'cable' is extremely tough and it is estimated that a collagen fibre 1mm in diameter would require a load of 10 kg to break it!

Individual α chains are assembled on ribosomes and then released into the rough endoplasmic reticulum (RER). It is here that the triple helix is assembled. An important additional modification at this stage is the hydroxylation of proline and lysine residues to hydroxyproline and hydroxylysine, a process that is vitamin C-dependent. Hydroxyproline is important in holding the immature helix within the RER and in maintaining its overall structural stability during temperature fluctuations. Hydroxylysine residues are necessary for α chain cross-linkage once collagen has been secreted into the extracellular space. Linkages typically align adjacent collagen fibres in a staggered fashion. Collagen synthesized when vitamin C levels are low is abnormal and severely affected individuals suffer from *scurvy* in which ulceration, wound breakdown and fragility of blood vessels are seen.

Collagenolysis is also important, not only in remodelling structural collagen but in degradation of collagen debris in wounded tissue. Hence, it is not surprising that procollagenase (the precursor of interstitial collagenase) is found in neutrophils, macrophages and fibroblasts. Collagenases are specific for different classes of collagen, with intersitial collagenase having substrate specificity for fibrillar or tissue collagens (types I, II and III). The second class of collagenases – the type IV collagenases – have substrate specificity for basement membrane collagen (type IV) and gelatins. The third class of collagenases are the stromelysins which degrade basement membrane constituents, including collagen types IV and V, proteoglycans, laminin, fibronectin and gelatin. These three types of collagenase are all members of an enzyme family which require zinc as a coenzyme – collectively known as the matrix metalloproteinases (MMPs). MMP activity is regulated by binding of small proteins known as tissue inhibitors of metalloproteinases (TIMPs).

Foetal healing

There are fundamental differences between the way foetal and adult wounds heal, with foetal skin healing rapidly with virtually no scarring. Foetal tissues are relatively hypoxic compared to adult tissues, are unable to mount a significant inflammatory response, and are bathed in sterile amniotic fluid, rich in growth factors. Simple manipulation of the adult wound environment to that of the foetus or transfer of wounded adult tissue *in utero* fails to induce the foetal phenotype, so the reasons for the difference must be more complex. One striking difference between foetuses and adults is the make-up of extracellular matrix. Foetal tissues have a much higher content of hyaluronic acid (HA) and a glycoprotein called hyaluronic-acid-stimulating activity (HASA). The extracellular matrix in healing adult tissues shows similar levels of HA only transiently before it is replaced by granulation tissue proper. High levels of HA appear to allow more freedom for cell migration and permit highly ordered collagen deposition throughout the wound. In addition to

Factors influencing tissue healing

Local factors

- Devitalized tissue
- Clot
- Foreign material
- Infection
- Tissue hypoxia:
  - Acute: vascular damage and suture tension
  - Chronic: radiation enteritis, diabetic microvascular disease, atherosclerosis, venous hypertension and long-term vascular damage

Systemic factors

- Nutritional deficiency (protein, zinc and vitamins A and C)
- Drug treatment (cytotoxic agents and glucocorticoids)
- Total body irradiation
- Hypercatabolic states (neoplasia, uraemia and jaundice)
- Hypoxia
- Diabetes mellitus

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HA, wounded foetal extracellular matrix contains more fibronectin and tenascin, which allow rapid epithelization.

Healing abnormalities

There are many local and systemic factors that contribute to impaired wound healing. In addition, there are situations where healing occurs but in a disorganized way (Figure 4).

Local factors pertain mainly to persistence of debris within the wound; for example, devitalized tissue, clot, foreign material, including sutures and bacterial contamination. These can act as a physical barrier to the ordered development of granulation tissues and collagen deposition, or may exaggerate the evoked inflammatory response. Ultimately, this can change the way tissues heal, changing primary to secondary union or resolution to organization. Local tissue hypoxia, either chronic (e.g. caused by radiation enteritis, atherosclerosis or diabetic microvascular disease) or more acute (e.g. caused by vascular damage or tight sutures) will compromise healing. Mobile wounds do heal but more slowly than those held together by sutures.

Systemic factors that impair healing include nutritional status, diabetes, glucocorticoid treatment, irradiation, hypoxia, jaundice and renal failure. Old age is a contentious issue: certainly wounds in older patients take longer to heal, but there are no studies to prove age correlates with a poorer quality of healing.

Several dietary deficiencies have been implicated in wound healing. As mentioned vitamin C (ascorbic acid) is essential for collagen synthesis. Apart from hydroxylation of proline, vitamin C is also required for production of n-acetyl galactosamine, a component of matrix and granulation tissue. Zinc deficiency impairs function of MMPs, which are essential for collagen and matrix remodelling. Any dietary state that limits the availability of amino acids will have a profound effect on healing and the quality of collagen made. Amino acids containing sulphydryl groups (e.g. methionine) are especially important.

Diabetic patients in whom sugar control is poor will suffer from a variety of problems affecting healing. First, high plasma sugar decreases both neutrophil chemotaxis and phagocytic ability. Additionally, a sugar-rich environment will favour microbial growth, further interfering with healing. Wounds in patients who have received high-dose glucocorticoids are known to heal poorly. This effect is probably due to the anti-inflammatory action of glucocorticoids as well as their direct depression of fibroblast collagen deposition.

Any condition that reduces blood flow and induces tissue hypoxia at the injured site will ultimately reduce healing (e.g. cardiorespiratory disease and sepsis). Conditions that reduce the number of lymphocytes or macrophages, or their ability to migrate, will also have a similar effect. Neoplastic cachexia, uraemia or jaundice all produce hypercatabolic states and will similarly interfere with healing.

There are occasions when abnormal healing occurs in an otherwise normal setting. Over-granulation (proud flesh) occurs when the margin of the granulation tissue protrudes above the margin of the wound. Subsequent epithelization will not occur in this situation and the granulation needs to be either cauterized with silver nitrate or surgically removed to allowing healing to occur. Other abnormalities can occur which result in ugly raised scars. Hypertrophic scars are limited to the wounded area and do not increase in size beyond 6 months. Keloid scars extend beyond the wound area and increase in size even after 6 months. Both abnormalities have excessive embryonic (type III) collagen within the scar, but only hypertrophic scars have increased collagenolysis. These abnormalities are commonly found in burn victims and black-skinned patients. There is no specific treatment for hypertrophic scars although excision and re-suturing is sometimes possible. Keloid scars have a tendency to recur following excision although intra-scar injection of steroids can be useful in this setting, stimulating collagenolysis and reducing fibroblast stimulation. ◆

FURTHER READING

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